USE OF A COMPOUND WHICH MODIFIES THE SECRETION OF INTERLEUKIN 5, INTERLEUKIN 6 AND/OR INTERLEUKIN 10 FOR THE PREPARATION OF A PHARMACEUTICAL COMPOSITION FOR TREATING ROSACEA

The present invention relates to the field of treating rosacea. The invention is directed towards providing novel pharmaceutical compositions, more particularly dermatological compositions, which are useful for treating rosacea and which comprise, as active agent, a modifying compound and/or a compound which inhibits the secretion of at least one interleukin chosen from the group comprising interleukin 5, interleukin 6 and interleukin 10.

Rosacea is a common, chronic and progressive inflammatory dermatitis associated with vascular relaxation. It mainly affects the central part of the face and is characterized by redness of the face or hot flushes, facial erythema, papules, pustules and telangiectasia. In serious cases, especially in men, the soft tissue of the nose may swell and produce a bulbous swelling known as rhinophyma.

Rosacea generally occurs between the ages of 25 and 70, and is much more common in people of fair complexion. It more particularly affects women, although this affection is generally more severe in men. Rosacea is chronic and lasts for years with periods of exacerbation and of remission.

Rosacea was originally called "acne rosacea" because its papules (points of slight raising of the skin) and its inflammatory pustules (pus scabs) greatly resemble those of common acne. In contrast with common acne, whose aetiology is based on abnormal keratinization, an increase in sebum production and also bacterial inflammation, the inflammation of rosacea is vascular in nature and is poorly understood. The result of this facial vascular anomaly is a permanent oedema of the

dermis, which may be accompanied by an increased colonization with *Demodex folliculorum*, a mite usually found in the follicles of the face.

According to various studies, *Demodex folliculorum* is thought to play an aetiological role in rosacea (Erbagi et al., 1998, Int. J. Dermatol., vol. 37, pages 421-425; Purcell et al., 1986, J. Am. Acad. Dermatol., vol. 15, pages 1159-1162; Sibenge et al., 1992, J. Am. Acad. Dermatol., vol. 26, pages 590-593). It appears that *Demodex folliculorum* causes or aggravates inflammatory reactions, reflected by papules and pustules, by blocking the pilosebaceous follicles of the face (Roihu et al., 1998, J. Cutan. Pathol., vol. 25, pages 550-552). This parasite is moreover thought to trigger a humoral immune response (Nunzi et al., 1980, Br. J. Dermatol. vol. 103, pages 543-551; Manna et al., 1982, Br. J. Dermatol, vol. 107, pages 203-208).

The pathogenesis of rosacea is poorly understood. Many factors may be involved without necessarily inducing this complaint. They are, for example, psychological factors, gastrointestinal disorders, environmental factors (exposure to sunlight, temperature, humidity), emotional factors (stress), dietary factors (alcohol, spices), hormonal factors or vascular factors, or even infection with *Helicobacter pilori*.

Rosacea develops in four stages, but passage through all the stages is not obligatory:

- stage 1 of vascular relaxations (at about 20 years old). The patients have sudden bursts of paroxystic redness of the face and neck, with a hot sensation, but with no systemic signs. After the attacks, the skin of the face returns to normal. These "flushes" are triggered by changes in temperature (occasionally leading to thermophobia), and the intake of hot drinks or alcohol;

- stage 2 of erythemato-telangiectasia (at about

30 years old). The cheekbone areas are diffusely red. Dilated capillaries constituting standard acne rosacea are observed therein. In contrast with stage 1, the redness is permanent. Besides the cheeks, the chin and the middle of the forehead may be affected;

- stage 3 of papulo-pustules (at about 40 years old). Papules and pustules a few millimetres in diameter develop on a background of erythema, without associated comedones. This dermatitis may be very extensive, occasionally up to the bald part of the scalp in men, but is absent from the area around the mouth and the eyes. The patients complain of sensitive skin, with subjective intolerance to the majority of topical products and greasy cosmetics;

- stage 4 of rhinophyma (at about 50 years old or later). This late phase mainly affects men, in contrast with the other stages. The nose is increased in volume and diffusely red, and the follicular orifices are dilated. The skin gradually thickens.

The minor forms of rosacea may be treated with active agents such as anti-seborrhoeic agents and anti-infectious agents, for example benzoyl peroxide, retinoic acid or metronidazole. Metronidazole, or 2-(2-methyl-5-nitroimidazolyl)ethanol, is known in the prior art for its antibacterial, antiparasitic and anti-protozoan properties. It exerts selective toxicity towards anaerobic microorganisms and also hypoxic cells. In the latter, metronidazole is reduced to derivatives capable of impairing the DNA structure of these cells.

As regards the most diffuse forms of the complaint, they respond well to general antibiotic therapy with cyclines. However, these treatments have unpleasant side effects for the patient, such as irritation or intolerance phenomena.

Furthermore, on account of the multi-factor aspect of

rosacea, there are a huge number of treatments for this condition, but the search is on again for an effective treatment that is without risk for the patient.

The Applicant's studies have demonstrated the involvement of certain interleukins in rosacea, and more particularly the involvement of interleukin 5, interleukin 6 and interleukin 10 in rosacea.

An infection with *Demodex folliculorum* triggers a humoral immune response. The humoral immune response involves activation of the type-2 T helper cells (Th2). Differentiation of the naïve T cells into Th2 cells is induced by interleukin 6. The Th2 cells then produce interleukin 4, interleukin 5 and interleukin 10, which stimulate activation of the B cells and the production of antibodies.

Interleukin 5 (IL-5), also known as "eosinophil colony stimulating factor", is secreted by the T lymphocytes. It may be classified among the growth factors of haematopoietic type since it stimulates the growth, differentiation and activity of eosinophils that play an important role in combating parasitic infections. Interleukin 5 also acts on eosinophils as a chemotactic agent. IL-5 induces the proliferation of the B lymphocytes and their secretion of immunoglobulins.

Interleukin 6 (IL-6), also known as "hepatocyte stimulating factor", "hybridoma growth factor" or "B cell stimulating factor", is a glycoprotein secreted especially by the T cells, monocytes and fibroblasts. It stimulates the growth and differentiation of the B lymphocytes into plasmocytes and increases the generation of platelets. It induces, via activation of the hepatocytes, the secretion of inflammation proteins such as fibrinogen and C-reactive protein. It has a pro-inflammatory role.

Interleukin 10 (IL-10) is produced by the T lymphocytes, the B lymphocytes and the mastocytes. IL-10 acts especially on the B lymphocytes: increase in the viability of the small B lymphocytes and increase in the expression of class II HLA molecules. This interleukin is also involved in regulating mastocyte proliferation.

The Applicant's studies have demonstrated the usefulness of compounds that modify the secretion of at least one interleukin chosen from the group comprising interleukin 5, interleukin 6 and interleukin 10 in the treatment of rosacea. This was observed by using metronidazole, the consequence of which is a change in the secretion of interleukins, and more particularly in the secretion of IL-5, IL-6 and IL-10. It has also been observed that the use of metronidazole had the consequence of inhibiting the secretion of interleukins, and more particularly the secretion of IL-5, IL-6 and IL-10.

As indicated previously, the invention is directed towards offering a novel method for treating rosacea, which consists in administering to an individual suffering from rosacea an effective amount of a compound that modifies and/or inhibits the secretion of at least one interleukin chosen from the group comprising IL-5, IL-6 and IL-10.

Consequently, the invention relates more particularly to the use of a compound that modifies, and advantageously inhibits, the secretion of at least one interleukin chosen from the group comprising IL-5, IL-6 and IL-10, for the preparation of a pharmaceutical composition for treating rosacea.

The invention also relates to the use of a compound that modifies and advantageously inhibits the secretion of two or three interleukins chosen from the group

comprising IL-5, IL-6 and IL-10, for the preparation of a pharmaceutical composition for treating rosacea.

As non-limiting examples of compounds that modify the secretion of at least one interleukin chosen from the group comprising IL-5, IL-6 and IL-10, mention may be made of the following compounds:

- Nisolpidine (Transpl. Int., 1992, 5, Suppl. 1: p. 398- p. 402),
- -SB203580, PD98059, U0216 (J. Immunol., 2002, 168: 861-868),
- Chloroquine (J. Immunol., 2000, 165: 1534-1540),
- 4-phenylthiazole derivatives and more particularly SCRC2941 (Bioorg. Med. Chem. Lett., 1999, 9: 957-960),
- 1-[6-((17beta-3-methoxyoestra-1,3,5(10)-thien-17-yl)amino)hexyl]-1H-pyrrole-2,5-dione, wortmannin, bisindolylmaleimide and bisindolylmaleimide X1 HCl (Ro-32-0432), 2'-amino-3'-methoxyflavone, 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1H-imidazole (Br. J. Pharmacol., 2003, 140; 764-772),
- Ro20-1724 and theophylline (Immunopharmacology, 1996, 31: 223-235), AS101 (J. Immunol. 2002, 169: 384-392).

More particularly, the pharmaceutical composition which is the subject of the present invention is a dermatological composition for topical application to the skin.

According to the present invention, the term "treating rosacea" means treating and/or preventing rosacea, at one or more of the stages described previously.

According to a first embodiment of the invention, the composition is for treating the first stage of rosacea.

According to a second embodiment of the invention, the composition is for treating the second stage of rosacea.

According to a third embodiment of the invention, the composition is for treating the third stage of rosacea.

According to a fourth embodiment of the invention, the composition is for treating the fourth stage of rosacea.

According to a preferential embodiment, the composition contains from 0.0001% to 20% of a compound as defined above, preferably from 0.1% to 2% of the said compound and more preferentially from about 0.75% to 1% of the said compound expressed by weight relative to the total weight of the composition.

Needless to say, the present invention concerns, besides the use of a compound capable of modifying and/or inhibiting the secretion of at least one interleukin chosen from the group comprising interleukin 5, interleukin 6 and interleukin 10, the use of derivatives thereof. The term "derivatives" means compounds that are distinguished from the said compound by substitution, addition or deletion of one or more chemical groups.

The invention also relates to a process for identifying a compound that inhibits the secretion of at least one interleukin chosen from the group comprising interleukin 5, interleukin 6 and interleukin 10:

a) placing the test compound in contact with peripheral blood mononuclear cells pretreated with concavalin A;
b) recovery of the culture supernatant;
c) measuring the amount of IL-5, IL-6 and IL-10 produced;
d) selecting the said compounds for which an inhibition of IL-5, IL-6 and IL-10 production is measured in the treated sample from step a) relative to the control value obtained with cells not placed in contact with the test compound.

Advantageously, the compositions of the invention comprise, besides a compound as defined above, at least one other therapeutic agent capable of increasing the efficacy of the treatment. Non-limiting examples of such agents that may be mentioned include antibiotics, antibacterial agents, antiviral agents, antiparasitic agents, antifungal agents, anaesthetics, analgesics, antiallergic agents, retinoids, free-radical scavengers, anti-priruginous agents, keratolytic agents, anti-seborrhoeic agents, antihistamines, sulfides, immunosuppressant products and antiproliferative agents.

According to one particular embodiment of the invention, the compound that modifies the secretion of at least one interleukin is not metronidazole. According to another particular embodiment of the invention, the composition of the present invention also contains metronidazole.

The compositions of the invention may also comprise any additive usually used in the pharmaceutical or dermatological field that is compatible with the compound as defined above. Mention may be made especially of sequestrants, antioxidants, sunscreens, preserving agents, for example DL-α-tocopherol, fillers, electrolytes, humectants, dyes, common mineral or organic acids or bases, fragrances, essential oils, cosmetic active agents, moisturizers, vitamins, essential fatty acids, sphingolipids, self-tanning compounds such as DHA, skin calmative and protective agents such as allantoin, pro-penetrating agents and gelling agents. Needless to say, a person skilled in the art will take care to select this or these optional additional compound(s), and/or the amount thereof, such that the advantageous properties of the composition according to the invention are not, or are not substantially, adversely affected.

These additives may be present in the composition in a proportion of from 0 to 20% by weight relative to the total weight of the composition.

Examples of sequestrants that may be mentioned include ethylenediaminetetraacetic acid (EDTA), and also derivatives or salts thereof, dihydroxyethylglycine, citric acid and tartaric acid, or mixtures thereof.

Examples of preserving agents that may be mentioned include benzalkonium chloride, phenoxyethanol, benzyl alcohol, diazolidinylurea and parabens, or mixtures thereof.

Examples of humectants that may be mentioned include glycerol and sorbitol.

The compositions of the invention may contain one or more pro-penetrating agents in preferential concentrations ranging from 0 to 20% and more preferentially ranging from 0.6% to 3% by weight relative to the total weight of the composition. Among the pro-penetrating agents that are preferentially used, without this list being limiting, are compounds such as propylene glycol, dipropylene glycol, propylene glycol dipelargonate, lauroglycol and ethoxydiglycol.

Advantageously, the compositions according to the invention may also contain one or more surfactants in preferential concentrations ranging from 0 to 10% and more preferentially ranging from 0.1% to 2%.

The compositions of the present invention may be in any galenical form normally used for topical application, especially in the form of aqueous, aqueous-alcoholic or oily solutions, dispersions of the lotion type, aqueous, anhydrous or lipophilic gels, emulsions of liquid or semi-liquid consistency of the milk type,

obtained by dispersing a fatty phase in an aqueous phase (O/W) or, conversely, (W/O), or suspensions or emulsions of soft, semi-solid or solid consistency of the cream, gel or ointment type, or alternatively microemulsions, microcapsules, microparticles or vesicular dispersions of ionic and/or nonionic type.

Preferably, the creams may be formulated from a mixture of mineral oil or from a mixture of beeswax and of water, which emulsifies instantaneously, to which is added the compound as defined above, dissolved in a small amount of oil such as almond oil.

The ointments may be formulated by mixing a solution of the compound as defined above in an oil such as almond oil in warmed paraffin, followed by leaving the mixture to cool.

As examples of compositions according to the invention, mention may be made of those comprising an active phase containing (expressed as weight percentages):

- 0 to 90%, preferentially 5% to 25% and especially 10% to 20% of water;
- 0 to 10%, preferentially 0 to 2% and especially 0 to 0.5% of surfactant;
- 0 to 20%, preferentially 0 to 10% and especially 2% to 5% of pro-penetrating agent;
- 0.0001% to 20% and preferentially 0.1% to 2% of the compound as defined above;

and an aqueous phase comprising a gelling agent, and water.

The aqueous phase of a composition according to the invention in the form of an emulsion may comprise water, a floral water such as cornflower water or a natural spring or mineral water chosen, for example, from eau de Vittel, the waters of the Vichy basin, eau d'Uriage, eau de la Roche Posay, eau de la Bourboule, eau d'Enghien-les-Bains, eau de Saint Gervais-les-

Bains, eau de Néris-les-Bains, eau d'Allevard-les-Bains, eau de Digne, eau de Maizières, eau de Neyrac-les-Bains, eau de Lons-le-Saunier, les Eaux Bonnes, eau de Rochefort, eau de Saint Christau, eau des Fumades, eau de Tercis-les-Bains, eau d'Avène and eau d'Aix-les-Bains.

The said aqueous phase may be present in a content of between 10% and 90% by weight and preferably between 20% and 80% by weight relative to the total weight of the composition.

Non-limiting examples that may be mentioned include gelling agents of the polyacrylamide family such as the sodium acryloyldimethyltaurate copolymer/isohexadecane/polysorbate-80 mixture sold under the name Simulgel 600 by the company SEPPIC, the polyacrylamide/C13-14 isoparaffin/laureth-7 mixture, for instance the product sold under the name Sepigel 305 by the company SEPPIC, the family of acrylic polymers coupled to hydrophobic chains, such as the PEG-150/decyl/SMDI copolymer sold under the name Aculyn 44 (polycondensate comprising at least, as components, a polyethylene glycol containing 150 or 180 mol of ethylene oxide, decyl alcohol and methylenebis(4-cyclohexyl isocyanate) (SMDI), at 35% by weight in a mixture of propylene glycol (39%) and water (26%)), and the family of modified starches such as the modified potato starch sold under the name Structure Solanace, or mixtures thereof.

The preferred gelling agents are derived from the polyacrylamide family, such as Simulgel 600 or Sepigel 305 or mixtures thereof.

The gelling agent as described above may be used in preferential concentrations ranging from 0.1% to 15% and more preferentially ranging from 0.5% to 5%.

The gels may preferably be prepared by dispersing or dissolving the compound as defined above in a suitable ratio in a gel of carbomer, poloxamer or cellulose-based type.

Other advantages and characteristics of the invention will emerge from the examples below concerning the activity of metronidazole as a compound that inhibits and/or modifies the secretion of at least one interleukin chosen from the group comprising IL-5, IL-6 and IL-10.

Example 1: Measurement of the secretion of interleukins

Materials and methods

The measurement of the secretion of IL-5, IL-6 and IL-10 was performed on peripheral blood mononuclear cells (PBMCS) according to the method used by Endo (Endo et al., 1993, Int. Arch. Allergy Immunol. vol. 101, pages 425-430). The PBMCs are isolated from heparin-treated peripheral venous blood, separated by density gradient centrifugation and suspended in RPMI 1640 medium. To stimulate the secretion of interleukins, the PBMCs are cultured in the presence of concavalin A at 20 μg/ml. The cells are then incubated for 48 hours at 37°C in the presence of metronidazole. The culture supernatant is then recovered and used to test the level of secretion of the interleukins. The productions of IL-5, IL-6 and IL-10 are quantified using enzymatic immunoassay kits (R&D System). The tests are performed in duplicate according to the manufacturer's recommendations. The results (Table 1) are expressed as a percentage of the control value and as a percentage variation of the control values.

The productions of IL-5, IL-6 and IL-10 by the PBMCs are studied in the presence of metronidazole at 10 μM.

Table 1

	Metronidazole (μM)	% of control value (± SD)
Secretion IL-5	10	73.7 ± 3.5
Secretion IL-6	10	69.2 ± 7.1
Secretion IL-10	10	76.6 ± 7.8

Metronidazole thus inhibits the secretion of interleukin 5, interleukin 6 and interleukin 10.